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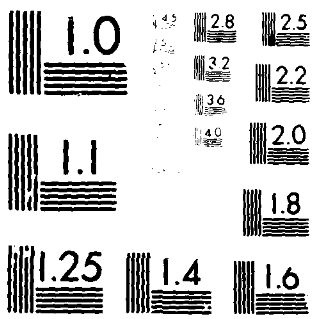

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TECHNICAL NOTE

MRL-TN-428

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F. John Upsher

Approved for Public Release

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MICROBIAL COLONIZATION OF MATERIALS AT INNISFAIL, QUEENSLAND

F. John Upsher

ABSTRACT

Materials were exposed under a glass canopy at the cleared site at Joint Tropical Trials and Research Establishment, Innisfail, Queensland. Two series of exposures were made; one starting in the cool dry winter, the other in the hot-wet season. Growth of microorganisms was slow, particularly of algae which were not apparent until 30 weeks; tardiness was attributed to the samples being protected from rain so that the organisms were dependent upon atmospheric moisture and dew. An increase in the amount of growth was apparent after any week in which the mean relative humidity exceeded 87% or when 80% was exceeded for more than 125 hours.

Cotton and wood provided the earliest growth and also supported the greatest amount and variety of fungi. Heavier growths were observed on acrylic paint and poly(vinyl chloride) after prolonged exposure. *Cladosporium* was the dominant fungal genus, being present on almost every occasion any fungus was detected.

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16. ABSTRACT *(if this is security classified, the announcement of this report will be similarly classified):*

Materials were exposed under a glass canopy at the cleared site at Joint Tropical Trials and Research Establishment, Innisfail, Queensland. Two series of exposures were made; one starting in the cool dry winter, the other in the hot-wet season. Growth of microorganisms was slow, particularly of algae which were not apparent until 30 weeks; tardiness was attributed to the samples being protected from rain so that the organisms were dependent upon atmospheric moisture and dew. An increase in the amount of growth was apparent after any week in which the mean relative humidity exceeded 87% or when 80% was exceeded for more than 125 hours.

Cotton and wood provided the earliest growth and also supported the greatest amount and variety of fungi. Heavier growths were observed on acrylic paint and poly(vinyl chloride) after prolonged exposure. *Cladosporium* was the dominant fungal genus, being present on almost every occasion any fungus was detected.

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C O N T E N T S

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MICROBIAL COLONIZATION OF MATERIALS AT INNISFAIL, QUEENSLAND

INTRODUCTION

It has been a constant observation that the hot-wet tropical climate favours microbial growth [1]. On porous inorganic materials, algae dominate but on organic materials, particularly those which are easily hydrolysed, fungi are generally more abundant, causing disfigurement and often deterioration.

In earlier studies of the fungal population at Joint Tropical Trials and Research Establishment (JTTRE, formerly Joint Tropical Research Unit), Innisfail, Queensland, attention was given to the airborne spores of saprophytic microfungi which included the types able to grow on materials [1]. Their occurrence patterns through the day and through the year were elucidated and their natural sources determined. However observations of microfungi on materials [2] have been less systematic; most have been made on specimens from trials in which assessment of microbiological growth was incidental to the intended purpose of the exposure.

In view of Australian deployment of Service materiel in hot-wet tropical areas, where microbiological spoilage is common, this investigation was undertaken as part of a programme concerned with service application of textiles, to study the colonization of some simple materials, noting the types of microfungi present, their successions, associations and affinities. Effects of tropical exposure on the physical properties of the test materials is not a part of this investigation, but the effects of microfungi on the surfaces of some materials will be presented separately.

EXPERIMENTAL

Materials

The following materials were used in the trial :

(1) Paint, acrylic white gloss. The formulation is given in Appendix A.

(2) Paint, alkyd white gloss. The formulation is given in Appendix A.

(3) Glass (window quality). Omitted from second series.

(4) Wood: white cheesewood, *Alstonia scholaris* was included in the 1st series but was not available for the 2nd series so *Pinus radiata* sapwood was substituted.

(5) Rubber, natural, cured with dicumyl peroxide.

(6) Poly(vinyl chloride) (PVC), plasticised. The formulation is given in Appendix A.

(7) Cotton duck, 400 g/m^2 loomstate.

(8) Polyethylene sheet, 0.05 mm, commercial.

(9) Cellophane sheet, 0.04 mm, lacquered.

Specimens

The specimens for exposure measured approximately 100 x 15 mm; the glass, rubber, PVC, polyethylene and cellophane were cut to that size from sheet materials. The paint was applied to unplasticised PVC strips by dipping. The wood was cut oversize and dressed down to give smooth surfaces. The cotton duck strips were cut oversize in the warpwise direction then frayed down to size and the edges trimmed.

Exposure

Specimens were clamped in aluminium frames (Figure 1) using 2-sided tape and polyethylene foam to ensure a grip, regardless of difference in thickness. Specimen frames were secured vertically facing north-south, under a horizontal glass canopy, to keep off rain and debris, at the open cleared site at JTTRE, Innisfail. The first series was put out on 6th June 1977 and the second series on 20th March 1978.

Meteorological data are presented in Appendix B as weekly summaries, for the duration of the whole exposure period.

Withdrawals

Specimen frames were withdrawn for examination after 1, 2, 3, 4 and 6 weeks, 2 and 3 months and less frequently after that (see Table 1). Series 1 exposures continued for 21 months and Series 2 for 12 months. Frames were securely packed to avoid abrasion of the surfaces of the specimens and were returned to Materials Research Laboratories (MRL) Melbourne for examination.

Examination

Assessments of microbiological growth and debris were made by visual and stereomicroscopic examination. Sporing structures and other taxonomic features were observed by microscopic examination and the presence of algae was determined by fluorescence microscopy.

Fungi were isolated from materials using a wet loop to transfer spores to several mycological media. Incidental spores, although seen, were disregarded since they did not constitute a part of the vegetative flora.

For comparative purposes detailed assessments were reduced to numerical ratings corresponding to the 0-5 scale described in Australian Standard 1157 [3] in which is a photographic reference plate; a total absence of growth or debris (as observed) is assessed as "0" through to a complete cover of a heavy deposit which would warrant a "5".

OBSERVATIONS AND DISCUSSIONS

General

Assessments of fungal growth and surface debris are presented in Tables 1 and 2 respectively and Figures 2-5 show materials in frames after exposure.

It is seen in Table 1 that a rating "1" can be followed by a "0". This is because a "1" must be applied to the minimum of actual growth, seen as hyphae radiating from a germinated spore, even if this does not become sustained but is merely transient superficial growth.

In the first series, in which exposure commenced in cool winter weather, rainfall was not heavy or persistent, humidities were correspondingly low, and fungal growth was slow to develop. Only the cotton fabric had any growth after 2 weeks exposure; PVC first showed growth after 3 weeks, wood after 4 weeks and only after 8 weeks did the acrylic paint, glass, and cellophane show their first growths.

Although the second exposure series was put out in the middle of the hot-wet season, there was no rain during the first week and daytime humidities were not high; consequently no growth appeared, but in the second week 62 mm of rain was recorded, the mean RH was 90% and 80% RH was exceeded for 133 hours, and fungi grew on wood, cotton fabric and cellophane.

As the wet season progressed, slight growth developed and persisted on all materials except the rubber and PVC, and it was not until the onset of the following wet season, and the 43-week withdrawal, that a marked increase in growth was noticeable, most obviously on the acrylic paint and PVC.

Debris accumulated on the surface of specimens throughout the exposure period, but deposition is thought to be greater during dry weather. In both series, the onset of drier July weather brought a general increase in the debris assessment rating. Most of the material deposited was mineral as seen in Figure 7.

Comparison of the weekly meteorological data (Appendix B), with weeks of significant fungal growth showed that humidity conditions were critical; growth occurred when the RH exceeded 80% for more than 125 hours or when the mean exceeded 87%. These figures suggest that it is the daytime humidity which makes the difference since during the hours of darkness the RH is almost always above 87%.

Rain would doubtless have been a greater influence in the development of microbial floras if it had been permitted to wet the specimens.

On those materials known to be readily susceptible to fungal attack, growth was apparent soon after a period of favourable humidities as seen in both exposure series - cotton after 1 and 2 weeks, timber after 4 and 2 weeks, cellophane after 8 and 2 weeks. The two paints and the PVC required a period of ageing and weathering and possibly also the accumulation of surface deposits before significant growth was apparent. The paints and PVC also showed a pronounced increase in the amount of growth during the wet season. Figures 6 and 7 show the alga *Desmococcus* from polyethylene and fungal hyphae from PVC respectively, both after 46 weeks exposure.

The growth on the glass was associated with wood fibres deposited from the adjacent specimen and other organic deposits; these may also have contributed to growth recorded on the rubber and polyethylene specimens.

In both exposure series, cotton showed an actual decrease in the amount of growth present after the first 8 weeks. This was probably due to the onset of drier weather during which growth became detached and possibly also to the increased resistance imparted to cotton by photochemical alteration during outdoor ageing as observed by Kaplan et al. [4].

Although known to be a highly susceptible substrate, the cellophane in this trial supported generally only slight fungal growth. This was considered to be due to the limited ability of this material to retain moisture - much less than the cotton duck and timber - which although of similar susceptibility are much bulkier.

The three cellulosic materials supported the greatest variety of fungi: cotton provided 21 genera, wood 18 and cellophane 15. The paints each supported 11, PVC 10, rubber 7 and polyethylene 6.

In this survey, a fungus was considered to be growing on a material when vegetative hyphae and recognisable sporing structures were observed on the material or when sporing structures were observed on colonies derived from mycelium on the material. After about 6 months exposure, the amount of organic debris on the exposed surfaces would be sufficient to support an appreciable part of the fungal flora but by the same token, the debris would also be feeding the fungi supported primarily by the substrate. In this investigation it appeared that *Paecilomyces*, which was observed on four materials after 97 weeks exposure (Table 3), was the only significant isolate to be sustained by surface deposits. Other genera recorded here are considered to have been growing on and at least partially dependent on the test materials for nutrients.

Cladosporium was found on all materials which supported any fungal growth and was present at most examinations; *Fusarium*, *Curvularia*, *Alternaria*, *Penicillium*, *Geotrichum*, *Rhinocladiella*, *Trichoderma* and *Paecilomyces* were isolated from more than a single type of material. The dominance of *Cladosporium* here concurs with its abundance in the air spora at JTTRE and elsewhere [1] and with previous findings on materials exposed there. Three species were frequently encountered, *C. sphaerospermum*, *C. cladosporioides* and *C. elatum*. Other morphological groups of *Cladosporium* were also isolated but have not yet been named.

Fusarium was present on nearly half of the cotton fabrics examined but was less prevalent on the other materials. Previously it had been considered to be associated more with the jungle, where its hyaline mycelium would be protected by the overhead jungle canopy from the lethal effects of solar radiation. Possibly the glass canopy over the specimens in this trial offered the same protection by filtering out some ultraviolet radiation.

Curvularia, although isolated from eight different materials, was only a regular member of cotton and cellophane floras. Two species, *C. eragrostidis* and *C. senegalensis*, were dominant. Although it had been found in air spora studies to be most abundant during the hot-wet season, it was present on materials throughout the year.

Epicoccum nigrum was notable not only as one of the first fungi to commence the colonisation of cotton but also for being the first to disappear; it was not detected after six weeks exposure.

Genera of fungi isolated from materials only occasionally during this trial included *Aspergillus*, *Aureobasidium*, *Bahusakala*, *Dreschlera*, *Geotrichum*, *Nigrospora*, *Paecilomyces*, *Penicillium*, *Phialophora*, *Phoma*, *Scopulariopsis*, *Scytalidium*, *Sporothrix*, *Sporotrichum*, *Stemphylium*, *Torula*, *Trichoderma* and *Trichocladium*. This is the first record of *Bahusakala* and *Scytalidium* on materials at JTTRE.

Algae were not apparent until 30 weeks exposure (Series 1) when *Desmococcus*, a green unicellular alga of the family Chlorococcales, appeared on the paints, cotton and glass at 46 weeks and later on wood and polyethylene. There was a subsequent increase in growth on the cotton and timber possibly because of their greater ability to retain moisture. The same algae were also present, associated with debris, on glass after 97 weeks exposure but there was no sign of the blue-green algae (Cyanophyta), *Scytonema stuposum* [5] and *Anacystis montana* [6], which had been observed on materials fully exposed to the weather at JTTRE. Normally green algae are more often seen on materials in the shade of the jungle than at the cleared site so presumably the glass canopy covering the exposed materials modified conditions sufficiently to disadvantage the cyanophytes and favour the chlorococcaceae.

PERFORMANCE OF THE MATERIALS

Without critical assessment of physical properties, the two paints, glass, wood and PVC appeared to have remained in good condition, beneath the surface deposits, throughout the exposure period.

The rubber showed surface crazing after four weeks, a condition which developed thereafter so that the surface was constantly contracting into tessellated islets, leaving freshly exposed material in between. Such microbial growth as developed was on the islets.

The cotton fabric became slightly frayed at the edges and the surface matted with wind-loosened fibres, but there was no obvious weakening.

The cellophane persisted well considering its innate fragility but tendered and fractured after about 30 weeks. The polythene fractured after 18 months - probably as a result of exposure to the sun.

CONCLUSIONS

Under the exposure conditions of this trial, fungi grew slowly. The cellulosic materials supported the earliest growth and the greatest variety of fungi. *Cladosporium* was the most widespread and *Curvularia* and *Fusarium* were also frequently encountered; green algae appeared on several materials after 30 weeks. Most critical to the storage of military material in tropical areas was the finding that most fungal growth occurred during weeks when the relative humidity exceeded 80% for more than when 125 hours or when the mean RH exceeded 87%.

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TABLE 1

ASSESSMENT OF FUNGAL GROWTH

SERIES 1

Exposure Period (weeks)	1	2	3	4	6	8	13	30	46	84	97
Acrylic Paint	0	0	0	0	0	1	0	1A	1	3	3
Alkyd Paint	0	0	0	0	0	0	1	1A	3	1	2
Glass	0	0	0	0	0	1	0	1A	1	2	2A
Timber (*Alstonia*)	0	0	0	1	1	1	3	2	2A	2A	2A
Rubber	0	0	0	0	0	0	1	0	0	1	1
PVC	0	0	1	1	0	0	1	0	2	2	4
Cotton	2	2	2	2	2	2	1	1A	2A	2A	2A
Polyethylene	0	0	0	0	0	0	1	1	1A	1	1
Cellophane	0	0	0	0	0	1	1	1	1	1	1

SERIES 2

Exposure Period (weeks)	1	2	3	4	6	9	13	20	33	43	52
Acrylic Paint	0	0	1	0	0	1	1	1	2	3	3
Alkyd Paint	0	0	1	0	0	1	1	2	1	1	2
Timber (*Pinus*)	0	1	1	1	1-2	2	2	3	2	2A	3A
Rubber	0	0	1	1	0	0	0	0	0	1	1
PVC	0	0	0	0	0	0	0	1	3	2	3
Cotton	0	1	1	1	2	1	1	1	1	1	2A
Polyethylene	0	1	0	0	0	1	0	0	1	1	1
Cellophane	0	1	1	1	1	1	2	1	2	1	1

Notes: 1. 'A' represents the presence of Chlorococcales (Algae).

2. Figures conform to the 0-5 scale described in AS 1157 in which 0 represents no growth through to 5 which represents total cover of a thick growth.

TABLE 2

ASSESSMENT OF DEBRIS

SERIES 1

Exposure Period (weeks)	1	2	3	4	6	8	13	30	46	84	97
Acrylic Paint	1	2	2	1	2	2	2	2	2	2	2
Alkyd Paint	1	1	2	2	1-2	2	2-3	3	2	2	2
Glass	1	1	2	2	2	1-2	2	2	2	3	2
Timber (*Alstonia*)	1	1	1-2	1	2	1	2	2	2	1	2
Rubber	1	2	1	1	2	2	2	2	2	2	2
PVC	1	1	1	2	2	1	2	2	2	2	2
Cotton	1	1	1	2	2	2	2	2	2	1	2
Polyethylene	1	2	2	2	2	2	2	2	2	2	2
Cellophane	1	2	2	2	2	2	2	2	2	2	2

SERIES 2

Exposure Period (weeks)	1	2	3	4	6	9	13	20	33	43	52
Acrylic Paint	1	1	1	1	1	1	1	1	2	2	2
Alkyd Paint	1	1	1	1	1	1	1	2	2	2	2
Timber (*Pinus*)	1	1	1	1	1	1	1	2	2	2	2
Rubber	1	1	1	1	1	1	1	1	1	2	2
PVC	1	1	1	1	1	1	1	2	2	2	2
Cotton	1	1	1	1	1	1	1	2	2	2	2
Polyethylene	1	1	1	1	1	1	1	2	2	2	2
Cellophane	1	1	1	1	1	1	1	2	2	2	2

Note: Figures conform to the 0-5 scale in AS 1157.

TABLE 3

OCCURRENCE OF MAJOR FUNGAL GENERA ON MATERIALS

	Cotton	Wood	Cellophane	Acrylic Paint	Alkyd Paint	PVC	Polyethylene	Rubber
Cladosporium	+	+	+	+	+	+	+	+
Curvularia	+	+	+	+	(+)	+	(+)	(+)
Fusarium	+	+	+	(+)		+	+	+
Alternaria	+	+				(+)		
Penicillium	+	(+)	(+)		(+)	(+)		
Paecilomyces	(+)		(+)	+			(+)	
Geotrichum	+	(+)				(+)		
Trichoderma	(+)			+				
Rhinocladiella		(+)	(+)					
Epicoccum	+							

(+) observed once only; + observed twice or more.

APPENDIX A

COMPOSITION OF MATERIALS

	Parts by weight
1. Paint, Alkyd, white gloss	
Long oil alkyd (Kemisol 3301)	2170
Rutile titanium dioxide (Rutiox RCR 3)	1200
Mineral turpentine	537
Naphthenate driers - Calcium	40
Lead	25
Cobalt	10
Manganese	2
2. Paint, Acrylic latex, white gloss	
Acrylic emulsion (Primal MV 1)	325
Rutile titanium dioxide (Rutiox RCR 3)	210
Methyl cellulose (Cellofas A, 5% in water)	63
Mica	42
Water	27
Plasticiser (dibutyl phthalate)	20
Sodium nitrite	12
Sodium benzoate	8
Dispersing agent (Calgon T, 5% in water)	6
Antifoaming agent	1
3. PVC - plasticised	
Poly(vinyl chloride) homopolymer (Corvic 20-6506)	100
Di(2-ethyl hexyl) phthalate	65
Octyl-9,10-epoxystearate (Lankroflex ED 3)	10
Calcium carbonate (Winnofil 5)	10
Tri-basic lead sulphate	2.5
Di-basic lead phosphite	2.0
Calcium stearate	0.5

APPENDIX B

WEEKLY METEOROLOGICAL SUMMARIES FROM THE CLEARED SITE, JTTRE, INNISFAIL (1977-79)

Withdrawals (weeks)		I/1	I/2	I/3	I/4		I/6		I/8			
Week Commencing	6.6	13.6	20.6	27.6	4.7	11.7	18.7	25.7	1.8	8.8	15.8	22.8
Mean R.H. (%)		76*				84*				75*		
Min. R.H. (%)	46	56^{+}	26^{+}	27	66	34	58	46	36	29	18	42
R.H. > 80% (h)	97	120^{+}	123^{+}	107	145	123	126	113	112	106	106	102
Rainfall (mm)	36	45	24	0	74	9	6	0	7	4	0	10^{+}

Withdrawals (weeks)		I/13										
Week Commencing	29.8	5.9	12.9	19.9	26.9	3.10	10.10	17.10	24.10	31.10	7.11	14.11
Mean R.H. (%)			80*				85*					78*
Min. R.H. (%)	40	40^{+}	26	30	43	49	47	20	49	41	44	49
R.H. > 80% (h)	124	99^{+}	83	113	110	102	106	77	95	78	81	84
Rainfall (mm)	30^{+}	4^{+}	0	29	58	3	26	0	6	0	0	0

APPENDIX B

(Continued)

Withdrawals (weeks)							I/30					
Week Commencing	21.11	28.11	5.12	12.12	19.12	26.12	2.1	9.1	16.1	23.1	30.1	6.2
Mean R.H. (%)				73*			83	87	88	86^{+}	83	79
Min. R.H. (%)	53	55^{+}	50^{+}	56^{+}	50	46	52	48	58^{+}	60^{+}	53	43
R.H. > 80% (h)	120	88^{+}	84^{+}	94^{+}	109	89	105	114	133^{+}	127^{+}	137	88
Rainfall (mm)	138	15	15	11	94	21	7	140	132	9	208	0

Withdrawals (weeks)							II/1	II/2	II/3	II/4	I/46	II/6
Week Commencing	13.2	20.2	27.2	6.3	13.3	20.3	27.3	3.4	10.4	17.4	24.4	1.5
Mean R.H. (%)	87	79	86	92	86	83	90	91	77	88	100	93
Min. R.H. (%)	59	57	59	70	43	51	54	64	34	48	58	66
R.H. > 80% (h)	118	129	113	143	118	103	133	152	105	106	136	150
Rainfall (mm)	95	74	18	114	195	0	62	440	1	3	53	169

APPENDIX B

(Continued)

Withdrawals (weeks)			II/9				II/13					
Week Commencing	8.5	15.5	22.5	29.5	5.6	12.6	19.6	26.6	3.7	10.7	17.7	24.7
Mean R.H. (%)	87	91	88	87	87	77	79	89	84	86	na	na
Min. R.H. (%)	63	49	55	57	58	32	35	55	43	50	na	na
R.H. > 80% (h)	125	142	130	128	121	99	130	132	117	127	na	na
Rainfall (mm)	271	26	9	24	2	23	0	4	65	0	1	6

Withdrawals (weeks)												
Week Commencing	31.7	7.8	14.8	21.8	28.8	4.9	11.9	18.9	25.9	2.10	9.10	16.10
Mean R.H. (%)	na	na	85	84	88	83	77	74	80	73	86	78
Min. R.H. (%)	na	na	41	33	53	46	32	47	47	19	42	38
R.H. > 80% (h)	na	na	105	119	125	114	93	91	98	72	121	99
Rainfall (mm)	8	42	3	75	25	10	3	1	1	14	182	26

APPENDIX B

(Continued)

Withdrawals (weeks)	II/33											
Week Commencing	23.10	30.10	6.11	13.11	20.11	27.11	4.12	11.12	18.12	25.12	1.1	8.1
Mean R.H. (%)	86	86	83	80	83	79	na	na	na	na	92	88
Min. R.H. (%)	48	61	54	47	45	46	na	na	na	na	59	60
R.H. > 80% (h)	104	129	101	103	110	na	na	na	na	na	150	128
Rainfall (mm)	56	83	15	2	78	na	na	na	na	na	723	163

Withdrawals (weeks)	I/84 II/43									I/93 II/52		
Week Commencing	15.1	22.1	29.1	5.2	12.2	19.2	26.2	5.3	12.3	19.3	26.3	
Mean R.H. (%)	87	89	90	91	90	88	87	95	82	78	85	
Min. R.H. (%)	58	58	60	65	61	62	46	46	70	40	31	
R.H. > 80% (h)	120	141	141	132	139	136	139	153	119	99	112	
Rainfall (mm)	176	175	290	335	336	83	84	386	6	6	17	

NOTES:
- * Monthly figures only available.
- \+ Data incomplete; best estimate given.
- na Data not available.
- I Represents First Series of Withdrawals.
- II Represents Second Series of Withdrawals.

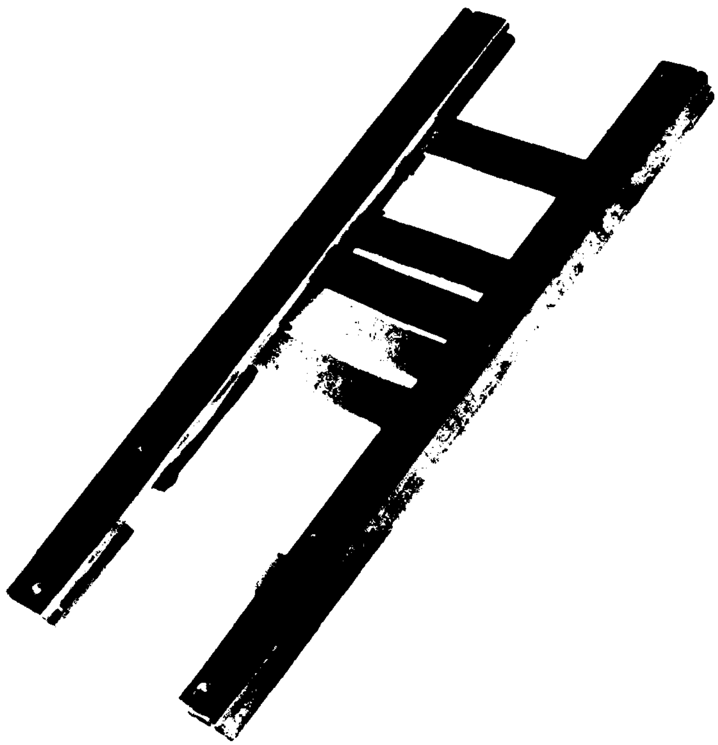

FIG. 1 - Exposure frame (Series 1) with specimens.

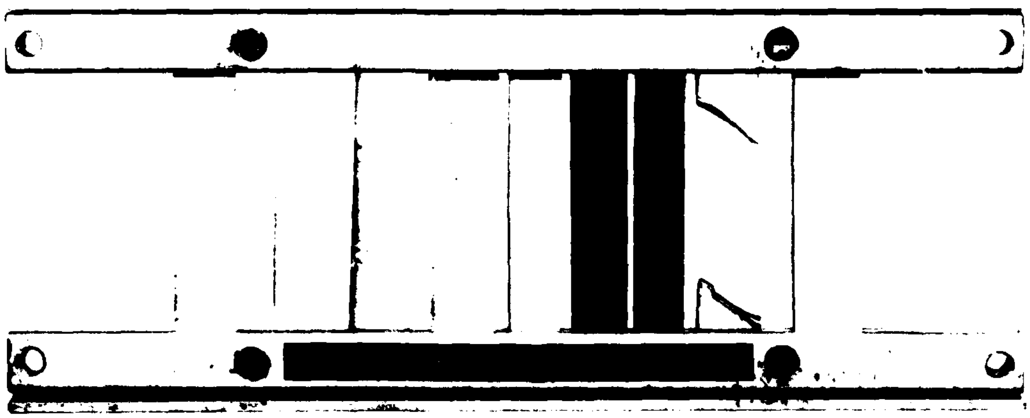

FIG. 2 - Series 1, after 12 weeks exposure.
Materials (left to right) - alkyd paint; cellophane; polyethylene; cotton; PVC; rubber; *Alstonia* wood; glass; acrylic paint.

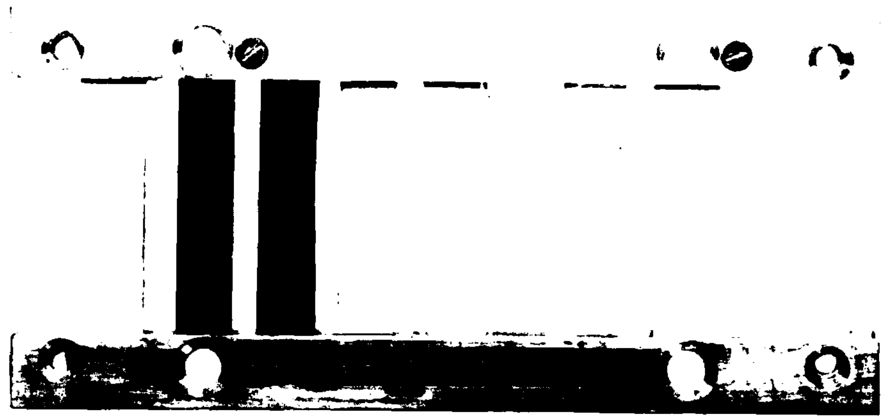

FIG. 3 - Series 2, after 14 weeks exposure.
Materials (left to right) - acrylic paint; *Pinus* wood; rubber; PVC; cotton; polyethylene; cellophane; alkyd paint.

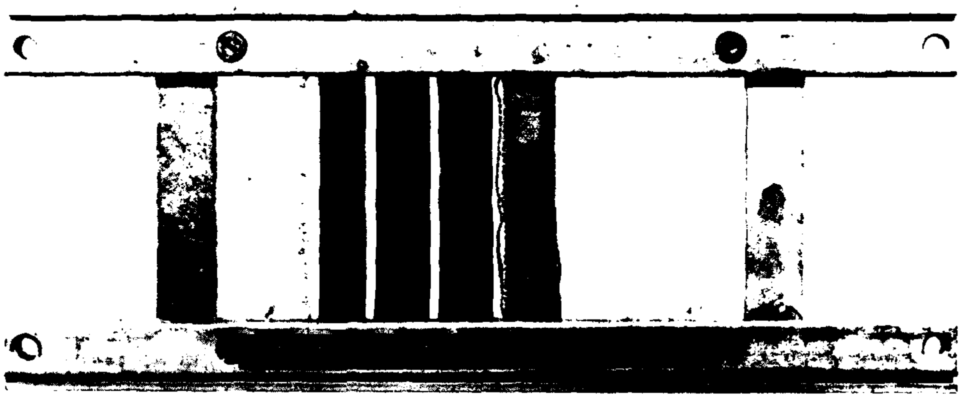

FIG. 4 - Series 1, after 18 months exposure.

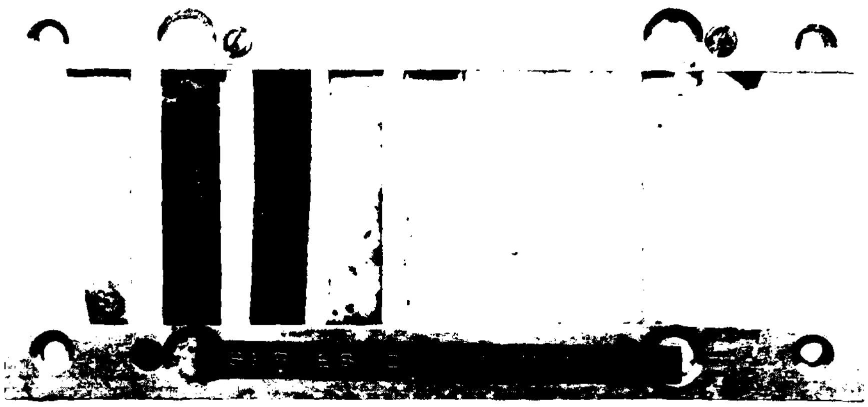

FIG. 5 - Series 2, after 12 months exposure.

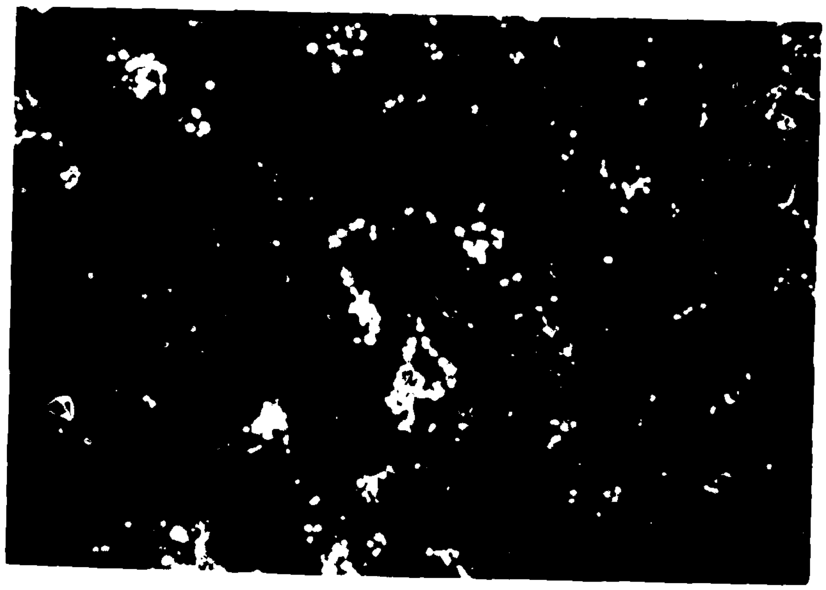

FIG. 6 - Deposits from surface of Polyethylene after 46 weeks exposure.
(Incandescent light, dark ground, x 250).
Major cellular component is the green alga *Desmococcus*.

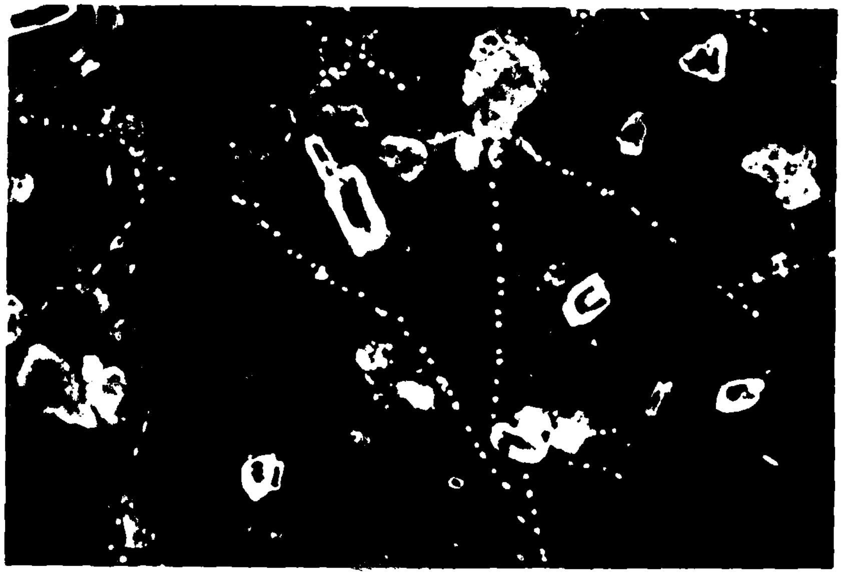

FIG. 7 - Deposits from surface of PVC after 46 weeks exposure.
(Incandescent light, dark ground, x 250).
Vacuolated hyphae are prominent; orange-brown particles are wind-blown mineral debris and large white-edged particles are glass from broken specimen.

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